ATOMIC RESOLUTION CRYOCRYSTALLOGRAPHY: BPTI AND CONCANAVALIN A

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Dramatic improvements in data quality for macromolecular crystals can be accomplished by cryogenic data collection. The advantages are manifold; problems are alleviated in almost all stages of the structure determination process. Firstly, the detrimental effects of radiation damage are postponed during data collection so that complete datasets can be collected from just one crystal, eliminating errors introduced by inter-crystal merging and scaling. Secondly, reduced thermal motion effectively increases the contrast in electron density maps, which facilitates interpretation. Suppressed radiation damage, reduced thermal smearing of electron density, combined with the possibilty of structural rearrangements to more ordered states can lead to substantially higher limiting resolutions which do not decay with time. We have recently completed low-temperature structural investigations of BPTI and concanavalin A.

In BPTI, flash cooling to 125 K induced small structural changes in the molecule which effectively locked in a single conformation for the two carboxyl terminal residues that had previously defied location in work at room temperature. In addition, the magnitude of disorder in side chains was reduced and over 90% of all solvent water was visible in electron density maps.

Cooled crystals of native concanavalin A invariably diffract x-rays to at least 1.2 Å and often beyond 1.0 Å. Data extending to 1.2 Å resolution have confirmed recent reassignments of several amino acids in the primary sequence and have revealed readily interpretable electron density in previously untraceable loop regions. The solvent region in these crystals is also considerably more ordered at low temperature.

The transition from room to low temperature in both these proteins have unusual changes in cell dimensions associated with them. In BPTI, the crystal a axis is longer at 125 K while in concanavalin A, a non-reversible, non-destructive, phase transition lengthens the b and c axes disproportionately with respect to the a axis between 160 and 165 K. Investigations into the nature of these strange temperature dependencies are ongoing.

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